Poll Res. 39 (February Suppl. Issue) : S142-S148 (2020)

ISSN 0257–8050

THE EFFECTS OF X BAND RADAR FREQUENCY EXPOSURE ON MICE TESTIS AND KIDNEY

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(Received 10 November, 2019; accepted 16 December, 2019)

ABSTRACT

There is a growing interest to investigate health hazards of exposure to electromagnetic fields (EMFs) on biological systems. Workers at transportation, telecommunications, medical and military fields are always exposed to EMFs. Testis and kidney diseases were demonstrated to be more common in these individuals than in personnel worked in other fields. The present study assessed the harmful effects of EMFs emitted by X band radar frequency on mice testis and kidney, by assessing their histopathological and morphological changes among two different age groups. A total of 30 male mice were assigned into three different groups: group I: unexposed group (control group), group II: two weeks age exposed group and group III: ten weeks age exposed group. A 9.5 GHz frequency with 80 mW power EMF was applied to group II and group III for 2 h per day for two months. The findings observed that the seminiferous tubules of exposed testis were degenerated compare with the control group. Also, the seminiferous tubule diameters were significantly reduced. while, the seminiferous tubule lumens of exposed groups were significantly increased. Histopathological and morphological alterations were also seen in mice kidney such as degeneration in the glomeruli and abnormal renal tubules. In addition, the glomerular surface area was reduced in both exposed groups compared with control group. In crux, the continuous exposure to EMFs can potentially cause detectable alterations on mice testis and kidney and that may lead to fertility problems and renal failure, respectively.

KEYWORDS: X band radar frequency, Histopathology, Histomorphometry, Testis, Kidney

INTRODUCTION

Radiation technologies such as mobile phone, microwave and radiofrequency (RF) have intensively used in the recent decades with the growing demand of electronic devices (Elhag, Nabil and Attia, 2007). X band radar frequency between 8-12 GHz is widely used in industrial, scientific, medical, military and domestic applications (Saygin *et al.*, 2011; Singh and Kapoor, 2015). Irradiation emitted by radar system into the environment may pose a health risk to the workers in certain occupations (Hao *et al.*, 2015). Since mobile phones and military radar devices are placed in close proximity to the genitals, the reproductive system is then absorbed high radiation rates when compared with other organs (Lavranos *et al.*, 2012). Several studies have reported the detrimental effects of EMFs on testis and seminal parameters, including motility, concentration and morphology (Tas *et al.*, 2014). Other studies investigated the cytotoxic effects of EMFs exposure on spermatogonia, spermatogenesis, induced apoptosis, decreased testicular biopsy score, degeneration of germ cells (Al-Akhras *et al.*, 2001; Aydin *et al.*, 2007; Khaki *et al.*, 2008; Khayyat, 2012). Whereas, other studies suggested that EMFs do not impact the male reproductive system (Heredia-Rojas *et al.*, 2004; Chung *et al.*, 2005). Similar experiments were conducted to investigate the EMFs effects on the kidney structure. They were found that the exposure EMFs can cause pathological effects in kidney tissue

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including glomerular degeneration and cytoplasmic vacuolization (Teimori *et al.*, 2016; Türedi *et al.*, 2017). Despite large number of studies regarding the harmful effects of EMFs, the histopathological and morphologicaleffects of X band radar frequency on testis and kidney are still controversial. Therefore, this work was designed to assess the adverse effects of EMFs on mice testis and kidney during two different age groups.

MATERIALS AND METHODS

Animals

This work was carried out at Physics Department, College of Science, University of Basrah. Thirty albino male mice were used in this study, they were obtained from the animal center in College of Veterinary/University of Basrah. The mice were housed under standard laboratory conditions with humidity and temperature-controlled in plastic ventilated cages. The system of illumination was designed to supply 12 h of day and 12 h of night conditions. Water and standard pellet food were not restricted. The study was approved by the Animal Research Ethical Committee of Basrah University.

X band radar frequencyexposure system

The mice were assigned into three different groups with ten animals in each group: group I (control group, unexposed to EMFs), group II (two weeks old group) and group III (ten weeks old group) were both exposed to EMFs. All mice of group II and group III were kept in ventilated cages during EMFs exposure while the control group remained unexposed in a separate room. In this study, microwave system with horn antenna (AT 3000 waveguide system, Atten electronics CO. LTD, China) was set up to produce X band radar frequency at 9.5 GHz frequency with 80 mW power. The antenna of the exposure system was oriented to the middle of the animal cage to supply an equal distribution of the EMFs. Following the duration of exposure, the mice were anesthetized by a piece of cotton soaked in chloroform in a plastic container in the package and then were sacrificed. Tissue samples were collected after and prepared for histopathological and histomorphometrical analyses.

Histopathological analysis

For histological examination, the testes and kidneys were carefully removed from each mouse after anesthesia. Tissue samples were fixed with 10% neutral buffered formalin solution in room temperature then processed through alcohol series (70%, 90%, 100%) and embedded in wax. Sections (5 µm thick) were cut, and dewaxed with xylene and rehydrated through a descending alcohol series. Slides were stained with hematoxylin and eosin (H&E). All of the stained sections were observed under light microscope.

Histomorphometrical analysis

ImageJ software (National Institutes of Health, USA), was used to evaluate the morphometric changes of mice testis and kidney after EMFs exposure. The external diameter of seminiferous tubules, their diameter lumen, and thickness of their epithelium (from the basement membrane to lumen) were estimated in H&E sections of mice testis. Also, ImageJ software was used to measure the glomerular surface area in H&E sections of mice kidney. All sections were calculated from *ca.* 10 tubules and glomeruli per testicular and kidney sections, respectively, and ten sections per group and subsequently averaged.

STATISTICAL ANALYSIS

Statistical analysis for the obtained data was performed using Prism statistical analysis software (GraphPad). All of the data with normally distributed are presented as the mean ± standard error of the mean (SEM). One-way analysis of variance (ANOVA) with Bonferroni's multiple comparison tests were used to analyze all data from the effects of EMFs on male mice. Statistically significant differences were accepted as $p < 0.05$.

RESULTS

Histopathologic and histomorphometric observations in mice testis after EMFs exposure

The histopathological examination of sections of testis of unexposed group showed normal spermatogenic and Sertoli cells in the seminiferous tubules and normal Leydig cells in the interstitial (intertubular) connective (Fig. 1A and Fig. 2A). In contrast, the sections of testis of group II revealed many histopathological changes. The seminiferous tubules were degenerated and the spermatozoa within their lumen were completely absence compare with the control group (Fig. 1B). Also, the

sections of testis of group III showed some of seminiferous tubules with irregular shape, a lower number of spermatozoa, detachments of spermatogonia from the basal membrane, increased cell distance and the presence of vacuoles in the epithelium of seminiferous tubule (Fig. 2B). The histomorphometrical analysis revealed a significant reduction in the diameter of the seminiferous tubules compared with the normal unexposed group (Fig. 3A). While, the seminiferous tubule lumens were increased (Fig. 3B). Also, the morphometric study of testis sections showed a reduction in the germinal epithelium thickness (Fig. 3C). However, there was no significant difference in the number of the seminiferous tubules per field between the control group and exposed groups (Fig. 3D).

Histopathologic and histomorphometric observations in mice kidney after EMFs exposure

Histopathological results of unexposed mice kidney revealed normal sections of glomeruli and renal tubules (Fig. 4A). In contrast, the exposed kidney sections showed abnormal glomeruli and renal tubules. The glomeruli were shrunken and atrophied and the renal tubules displayed many pyknotic nuclei and cytoplasmic vacuolation in their epithelial cells (Fig. 4B and C). Also, the histomorphometric analysis revealed a reduction in the glomerular surface area of both exposed groups compared with unexposed group (Fig. 4D).

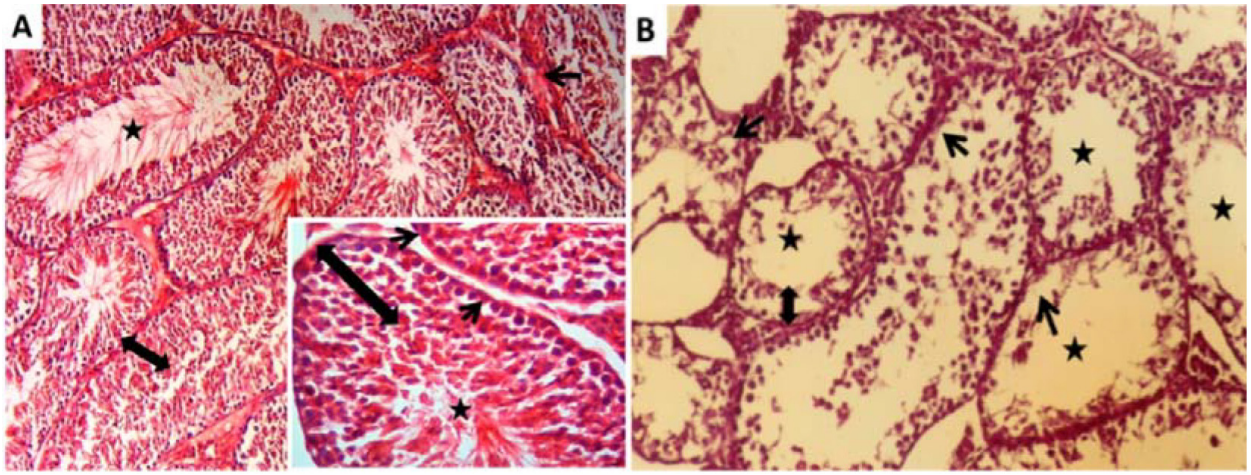

Fig. 1. Histopathological changes in group II mice testis. (A) Photomicrograph representing control group with normal testicular tissue. (B) Photomicrograph of group II showing degeneration in seminiferous tubules, no sperms in the lumen of seminiferous tubules (stars). Sections were stained with H&E, magnifications of main images: x200; inset x400.

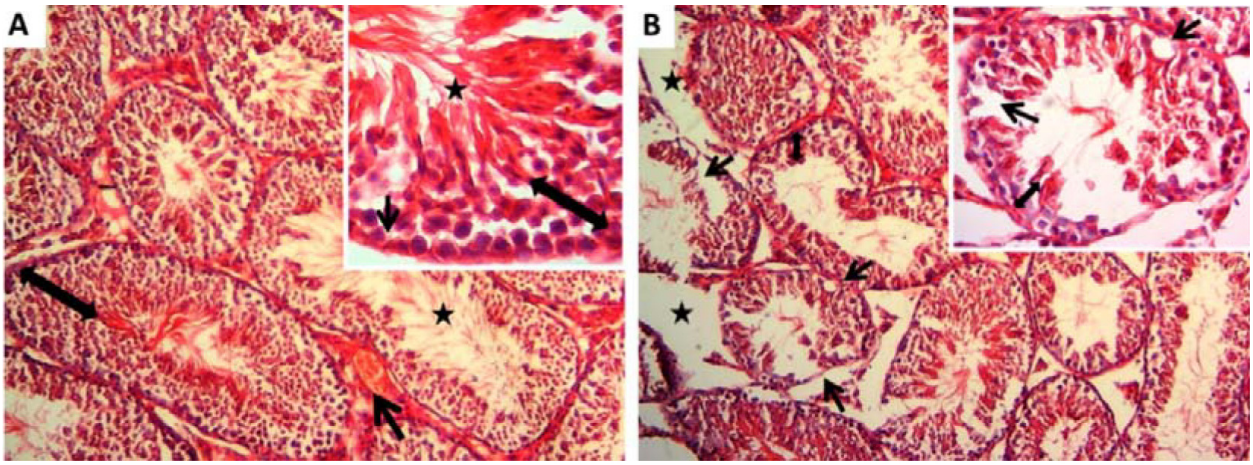

Fig. 2. Histopathological changes in group III mice testis. (A) Photomicrograph of group I showing normal testicular tissue. (B) Photomicrograph of group III showing degenerated and disorganization of some seminiferous tubules. Sections were stained with H&E, magnifications of main images: x200; inset x400.

DISCUSSION

X band radarfrequency is widely used in transportation, telecommunications, medical and militaryfields(Saygin *et al.*, 2011; Singh and Kapoor, 2015). Therefore, the present study was aimed at evaluating the toxic effects of EMFs emitted by X band radar frequency on histology and morphology of the testis and kidney in male mice. The effect of EMFs on the malereproductive and renal systems is remaining the topic of serious controversy.Based on the results of the present work it seems that the EMFs leads to significant alterations in the testicular tissue such as degeneration in the seminiferous tubules with reduction in the amount of spermatozoa within the lumen in both exposed groups. The morphological analysis was also performed to determine whether there was any influence of EMFs on testis morphology. The results showed a great number of morphological deteriorations such as a reduction in the seminiferous tubules diameter and an increased in their lumen compared with the unexposed group. Also, the germinal epithelium thickness was significantly reduced. Previous *in vitro* study was found that the direct effect of RF caused reduction of the fertilization of the irradiated epididymal sperm without any changes on the morphology of the spermatozoa (Cleary *et al.*, 1989). Also, another study showed different statistical results in the percentage of Leydig cells in the interstitial tissue

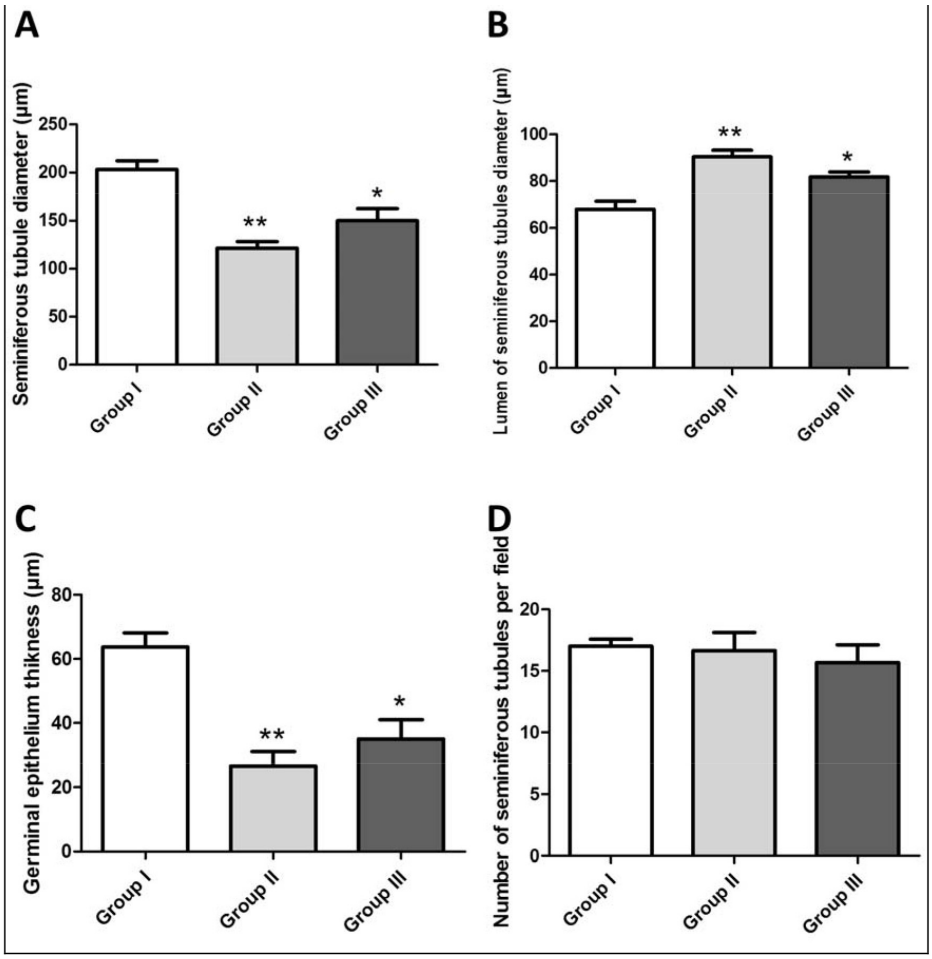

Fig. 3. Histomorphometric analyses of seminiferous tubules in mice testes. (A) Bar chart displaying the seminiferous tubules diameter across conditions. (B) Bar chart showing lumen diameter across conditions. (C) Bar chart showing germinal epithelium height across conditions. (D) Bar chart representing the number of seminiferous tubules per field across all conditions. One-way ANOVA, Bonferroni's post-test with error bars representing SEM. *P < .05 and **P < 0.01 versus unexposed group.

and in the weight of both germinal epithelium and testis tissue following the exposure to 900 MHz of EMFs (Ozguner *et al.*, 2005). The present results are compatible with pervoius study which showed that the EMFs exposure can damage the testis and the Leydig cells (Saunders and Kowalczuk, 1981a,b). Kumar *et al*. reported a reduction in total sperm count after EMFs exposure (Kumar *et al.*, 2014). The findings are also compatible with previous researches findings, which indicate that EMFs affected testicular function, disturb spermatogenesis and may cause infertility and reduce diameter of seminiferous tubular in rat (Saygin *et al.*, 2011; Tenorio *et al.*, 2012; Tas *et al.*, 2014). EMFs impact on tissues by producing heat (Black and Heynick, 2003). Importantly, a few degrees centigrade more than normal body temperature reduce sperm generation. Therefore, the testes are damaged by the heat generated after EMFs exposure (Saunders and Kowalczuk, 1981a). Therefore, longer or more powerful exposures may yield more effects, which might warrant further study. In contrast, previous experiment studied the effects of the RF on rat testes, the researches applied EMFs with frequency 2.45 GHz and specific absorption rates value 1.4 W/kg during two months (Moon *et al.*, 2007). They found the exposure to RF did not have any noticeable effects on rat spermatogenesis seminiferous tubules diameter, the number of epididymal sperm, spermatagonium cells and the motility of the sperms. Similar study was found that EMFs exposure have no effects on male reproductive system (Tumkaya *et al.*, 2016). Further, EMFs effects was investigated on kidney structure,

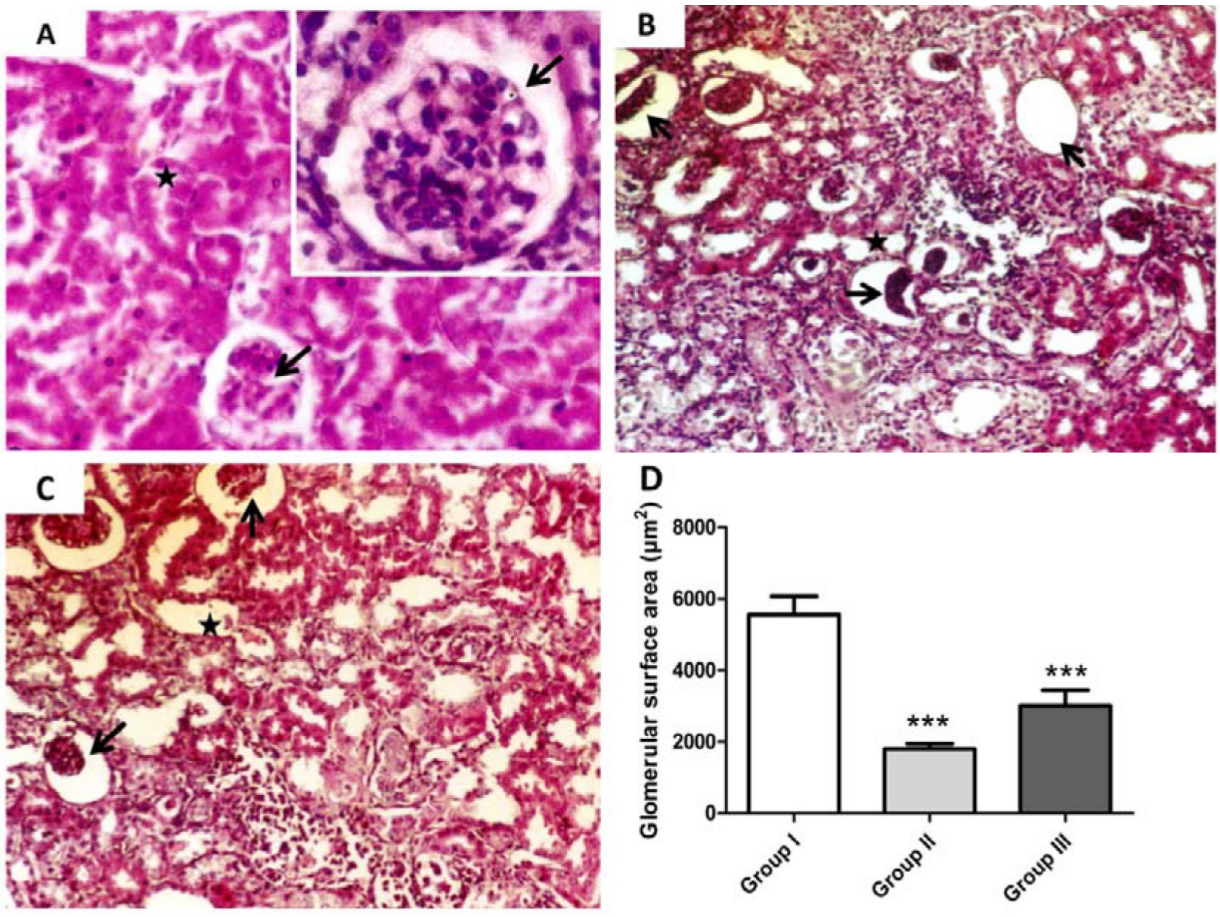

Fig. 4. Histopathologic and histomorphometric analyses in mice kidney. (A) Photomicrograph showing normal glomerulus and renal tubules in the control group. (B) and (C) Photomicrographs showing abnormal kidney structure in group II and group III, respectively. (D) Bar chat representing the surface area of glomeruli. Sections were stained with H&E, magnifications of main images: x200; inset x400. One-way ANOVA, Bonferroni's post-test with error bars representing SEM; ***P < 0.001 versus control group.

the present findings showed degeneration in glomerulus,and vacuolization in tubular epithelium cells in the kidney tissue of mice in both EMFs exposed groups. The tubular and glomerular damage plays vital role in acute renal diseases (Koca *et al.*, 2013). In parallel to histological analysis, the histomorphometrical analysis revealed a reduction in the glomerular surface area of both exposed groups compared with unexposed group. Some previous animal studies have reported that different frequencies and durations of EMF can cause pathological effects in kidney tissue such as glomerular atrophy, cytoplasmic vacuolization and pyknotic nuclei in the renal tubule epithelium (Ozturk *et al.*, 2003; Fahmy *et al.*, 2015). Another study suggested that the exposure to EMFs during pregnancy caused degeneration in the kidney tubule epithelium on a postnatal day 21 in rat pups (Odaci *et al.*, 2015). Also, they were observed a reduction in the number of glomeruli and the volume of cortex-medulla following EMFs exposure (Ulubay *et al.*, 2015). In addition, previous report emphasized that exposure to EMF can result in glomeruli and tubules injuries and dilation and impairment in Bowman's capsule (Koca *et al.*, 2013). Depending on the present study findings the EMFs produced serious histopathological and morphological alterations in mice testis and kidney. Since exposure group II comprised higher adverse effects as compared with groups III. Several studies suggest that children and teenagers may be significantly more sensitive than adults to the effects of EMFs (Kumlin *et al.*, 2007). Conflicting results have been reported regarding the toxic effects of radar bands on testis and kidney diseases. The variations in the results might be attributed to variation in the levels of exposure such as frequency, time of exposure, the difference in the experimental design.

CONCLUSIONS

Based on the present results, it was concluded that exposure of male mice to a 9.5 GHz frequency with 80 mW power EMFs for 2 h per day for two months did produce detectable alterations in testis and kidney and these alterations may lead to fertility problems and renal failure, respectively. However, further supporting research is definitely needed on this topic to understand the mechanism of EMFs effects on mice tissues. Furthermore, more attention should be paid to the effects of X band radar frequency with different frequencies and exposure periods on kidney and testicular function.

ACKNOWLEDGEMENTS

Authors would like to expresses their gratitude towards University of Basrah for its continuous support and encouragement during this work.

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